# Field Responses of *Anopheles gambiae* Complex (Diptera: Culicidae) in Liberia using Yeast-Generated Carbon Dioxide and Synthetic Lure-Baited Light Traps

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**ABSTRACT** Malaria infection is a serious public health problem throughout Liberia, but vector surveillance is limited or nonexistent in remote regions of the country. To better understand the spatial and temporal distribution of malaria vectors in Liberia and to support vector and malaria activities of the Liberian Ministry of Health, a study was conducted to determine the efficacy of light traps baited with a synthetic lure and $CO_2$ for capturing *Anopheles gambiae sensu lato* (Giles). Traps with a ultraviolet, light-emitting diode, and incandescent lights baited with a synthetic skin lure and $CO_2$ combinations were evaluated at four field sites in three counties of Liberia for five consecutive nights every 8 wk during 2011. In total, 4,788 mosquitoes representing 56 species from nine genera were collected throughout the 30-wk study; *An. gambiae s. l.* comprised 32% and of the148 *An. gambiae s. s.* collected, 85% were of the S form. A greater percentage of *An. gambiae s. l.* were collected in ultraviolet traps baited with a synthetic lure and $CO_2$ compared with any other trap configuration. The influence of trap configuration on conclusions from surveillance efforts, specifically with regards to *An. gambiae* is discussed.

**KEY WORDS** mosquito, UV, light-emitting diode, Malaria, S-form

The *Anopheles gambiae sensu lato* Giles complex is made up of six morphologically similar mosquito species, differentiated by a unique set of habitat, behavior, and feeding preferences (Coetzee et al. 2000). In Liberia, this complex is made up of *Anopheles gambiae sensu stricto* (Giles), *Anopheles melas* Theobald and possibly *Anopheles arabiensis* Patton, although the presence and distribution of the latter member remains unclear. Successful surveillance for this mosquito complex can be difficult in the field, as members are not only attracted to several hosts, but are also influenced by varying degrees of attraction to the primary host (Costantini et al. 1999). For example, although some members of this complex are endophilic, segments of the same population, as well as other species within the complex, are exophilic, often requiring multiple surveillance techniques to adequately determine their distribution and abundance (Service 1993).

*An. gambiae s.s.* exhibits strong anthropophilic, endophagic, and endophilic behavior, making it a highly efficient human malaria vector, with distribution throughout sub-Saharan Africa (Takken and Knols 1999). *An. gambiae s.s.* has also undergone sympatric ecological diversification forming two incipient species known as "M" and "S" molecular forms, whereby the M-form demonstrates a greater ability to exploit breeding sites created by human activity (Caputo et al. 2011). *An. gambiae s.s.* distribution in relation to human habitation plays an important role in their potential as malaria vectors and any malaria control program. This is especially true for the M-form, which has adapted to breed throughout the year in habitats around human settlements, extending malaria transmission from seasonal to year-round (Caputo et al. 2011).

Common surveillance methods used to sample *An. gambiae* include human landing counts, pyrethrum spray catches, and light, bed-net, tent, and odor-baited traps (Mboera 2005). The Centers for Disease Control and Prevention (CDC) light trap with its typical 4–6 W incandescent bulb remains a standard adult mosquito surveillance tool having proved effective for trapping *An. gambiae* inside and within immediate areas surrounding human dwellings (Odetoyinbo 1969, Joshi et al. 1975, Mbogo et al. 1993, Githeko et al. 1994). However, trapping inside houses may bias surveillance results by excluding exophilic mosquito spe-

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14. ABSTRACT

**Malaria infection is a serious public health problem throughout Liberia, but vector surveillance is limited or nonexistent in remote regions of the country. To better understand the spatial and temporal distribution of malaria vectors in Liberia and to support vector and malaria activities of the Liberian Ministry of Health, a study was conducted to determine the ef?cacy of light traps baited with a synthetic lure and CO2 for capturing Anopheles gambiae sensu lato (Giles). Traps with a ultraviolet, light-emitting diode, and incandescent lights baited with a synthetic skin lure and CO2 combinations were evaluated at four ?eld sites in three counties of Liberia for ?ve consecutive nights every 8 wk during 2011. In total, 4,788 mosquitoes representing 56 species from nine genera were collected throughout the 30-wk study; An. gambiae s. l. comprised 32% and of the148 An. gambiae s. s. collected, 85% were of the S form. A greater percentage of An. gambiae s. l. were collected in ultraviolet traps baited with a synthetic lure andCO2 compared with any other trap con?guration. The in?uence of trap con?guration on conclusions from surveillance efforts, speci?cally with regards to An. gambiae is discussed.**

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cies responsible for outdoor malaria transmission in a particular region. Moreover, the attractive range of light from a CDC trap targeting *An. gambiae* is no >5 m from human dwellings (Odetoyinbo 1969) and placing traps at this distance is often not feasible to owing to the possibility of theft and or cultural suspicion and fears.

Human landing collections are the "gold standard" for determining entomological inoculation rates and sampling the primary anthropophagic mosquitoes within an area, yet this method is labor intensive, dangerous to the volunteer, and can produce inconsistent results owing to variability in attractiveness among collectors (Mboera 2005). Unlike many mosquito species that detect and locate hosts through $CO_2$ concentration gradients, the anthropophilic attraction of *An. gambiae s. s.* is due, in part, to human-specific volatile compounds (Mboera and Takken 1997, Costantini et al. 1999), emanated by human skin bacteria (Verhulst et al. 2011). These kairomones can have synergistic properties, where attraction is magnified in the presence of another. For example, ammonia, lactic, and carboxylic acids have shown to elicit a greater host-seeking behavior response of *An. gambiae*, than when evaluated alone (Smallegange et al. 2005). Furthermore, differing $CO_2$ concentrations and non-$CO_2$ bait combinations are known to attract different members of the *An. gambiae* complex (Dekker and Taken 1998).

Although *An. gambiae* response to "attractive" odors has been well-documented in olfactometers and laboratories and under indoor and semifield conditions, few studies describe their effects on trap captures in field settings. Replicating laboratory and indoor mosquito responses to attractants in the field is difficult. Discrepancies between field and laboratory may be a result of prolonged maintenance of mosquito colonies under artificial conditions and their strong propensity to become conditioned to physical and chemical cues from human hosts during husbandry activities (Njiru et al. 2006).

Most previous studies of trap efficacy in malarious regions have placed traps inside human dwellings, potentially neglecting exophilic anopheline species, and few have investigated attractants in combination with newly available light trap technology. Combining known olfactory attractants with mosquito light traps that emit a specific wavelength or color may enhance mosquito surveillance by attracting specific mosquito species from a greater distance. It has been reported that light traps baited with olfactory cues collect particular species from a greater area than $CO_2$ alone (Gillies and Wilkes 1970). Therefore, magnifying the attraction over an area may assist agencies in targeting regions that are not sampled by conventional surveillance methods. In addition, light traps need to be energy efficient, especially in remote regions, where access to electrical power is often limited. The development of light-emitting diodes (LEDs) offers many advantages over other light types used in mosquito traps, the greatest being that LEDs are at least three times more energy efficient compared with incandescent and fluorescent light bulbs (Cohnstaedt et al. 2008). Finally, adult mosquito surveillance in many rural areas is challenging owing to a lack of $CO_2$ accessibility either in the form of dry ice or compressed gas.

As part of a larger investigation to determine the distribution of potential vectors responsible for malaria transmission in the region and to determine the most effective light trap to collect *An. gambiae*, we evaluated the efficacy of three trap types that use different light sources and baited these with various combinations of a synthetic lure and a yeast–sugar mixture as a source of $CO_2$. Our objectives were to (1) determine the efficacy of multicombination baited lights traps in capturing *An. gambiae* in field settings and (2) identify members of the *An. gambiae* complex as well as molecular forms of *An. gambiae s. s.* collected.

## Materials and Methods

### Study Area

Adult mosquito surveillance was conducted every 8 wk from January to November, 2011. Traps were placed in four sites, encompassing three counties (Fig. 1). Sites 1 and 3 were in Margibi county, within 4 km of the coast, while site 2 (Montserrado County) was located 25 km from the coast. Site 4 was situated in Lofa County, the northern most county in Liberia that borders Guinea. Sites were selected based on habitat selection and security concerns. Site 1 (N 06° 46.53, W 010° 51.50) contained numerous sand dunes interspersed with low growing vegetation and coconut palm trees (*Cocus nucifera* L.). Immediately adjacent to site 1 was a large brackish swamp bordered by mangrove trees (*Rhizophora mucronata* Lamarck). Site 2 (N 06° 47.00, W 010° 47.76) contained numerous tropical plants mainly silk cotton tree (*Ceiba pentandra* L.), giant bamboo (*Bambussa oldhamii* Munro), and groves of cassava (*Manihot esculenta* Crantz). Site 3 (N 06° 12.28, W 010° 22.51) was located on Liberian Institute of Biomedical Research (LIBR) property, which was adjacent to rice fields and a rubber tree plantation. Site 4 (N 08° 23.59, W 09° 42.57) was located at the LIBR-Lofa facility, adjacent to a forest.

### Traps, Settings, and Baits

Three kinds of light traps were used in the study: (1) the CDC miniature light trap (model 512, John W. Hock Company, Gainesville, FL), (2) a 4-W miniature UV CDC light trap, and (3) a UV LED light trap (Bioquip Products, Rancho Dominguez, CA). The CDC light trap contains an incandescent white light bulb, while a UV fluorescent bulb and eight LED elements are used in the UV and LED traps, respectively. A black 33 cm ABS trap cover (Bioquip Products, Rancho Dominguez, CA) was substituted for all trap covers, ensuring consistency among traps and maximizing efforts to protect trap

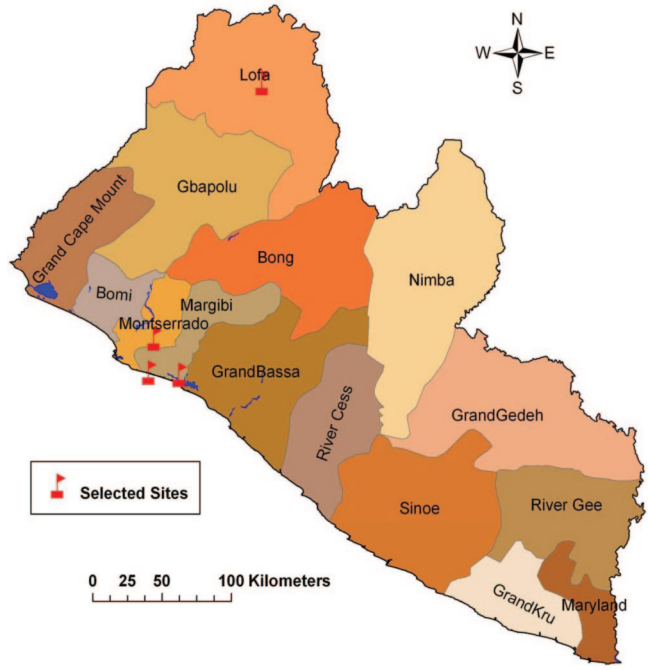


**Fig. 1.** County map of Liberia depicting the four mosquito surveillance sites used in this study. (Online figure in color.)

catches from drenching rains. All traps were powered by six V rechargeable gel cell batteries (UKB Co, LTD, Korea).

To increase *An. gambiae* trap attraction, a synthetic lure (BG-Lure) (Biogents, Germany) was attached to the light trap housing unit using paperclips. Although primarily developed and used to attract day flying *Stegomyia* (*Aedes*) mosquitoes, blends of this lure's primary ingredients (ammonia, lactic, and aliphatic carboxylic acids) have been shown to increase trap efficacy for *An. gambiae* (Murphy et al. 2001, Smallegange et al. 2005). Lures were replaced after five consecutive nights, ensuring a fresh lure for each trial.

Carbon dioxide was generated using a combination of brewer's yeast, sucrose, and water following similar methods by Smallegange et al. (2010). At the start of each trapping cycle, premeasured bags containing 35 g of yeast and 250 g of sugar were placed in a 10-liter plastic jug with 2.5 liters of water and shaken. A plastic cap was used to seal the contents, while a plastic screw cap secured the inner cap to the plastic jug, providing the final seal and ensuring an air-tight fit. Carbon dioxide was delivered from the container to the trap using 6.4-mm-diameter black plastic tubing (Clarke Mosquito Control and American Biophysics, Roselle, Illinois) and secured to the trap top with a rubber band (Fig. 2). A thin layer of petroleum jelly was applied to all metal poles, tubing, and wires to prevent ants from consuming trapped mosquitoes. At each of the four sites, traps were placed along a transect, spaced ≈30 m from each other, and mounted on a 2-m metal pole, suspended 1.5 m above ground level. This distance was selected owing to a Gambia study demonstrating a minimum distance of 14–18 m between host stations was ideal to avoid host-cues mixings from different sources (Gillies and Wilkes 1970). Traps were set at 1700 hours and collected next morning at 0700 hours, constituting one trapping period. Traps were set for five consecutive nights every 8 wk (one trial). All seven trap combinations were run simultaneously at each site. Each night, the position of each trap (treatment) at each site was randomized along the transect, to address any within-site bias.

### Specimen Identification and Molecular Analysis

After traps were collected, the catch was transported to the lab and mosquitoes were frozen at −20°C for 3 h, enumerated and dispensed into 2.0-ml plastic micro-centrifuge tubes containing cotton and dry silica gel for preservation. Specimens were transported and identified at the U.S. Naval Medical Research Unit no.3, Cairo, Egypt. Anopheline adults were morphologically identified using dichotomous keys of Stojanovich and Scott (1966), and other mosquito species were identified using Edwards (1941). After identification, the heads and abdomens of known anopheline malaria vectors were excised using a scalpel and tested for proteins of *Plasmodium falciparum* Welch using enzyme-linked immunosorbant assay (Wirtz et al. 1987). Members of *An. gambiae* were identified by polymerase chain reaction; specimen legs were excised and subjected to DNA extraction using QIAGEN DNA Mini Kit (QIAGEN, Valencia, CA), stored at −20°C, and identified using ribosomal DNA–polymer-

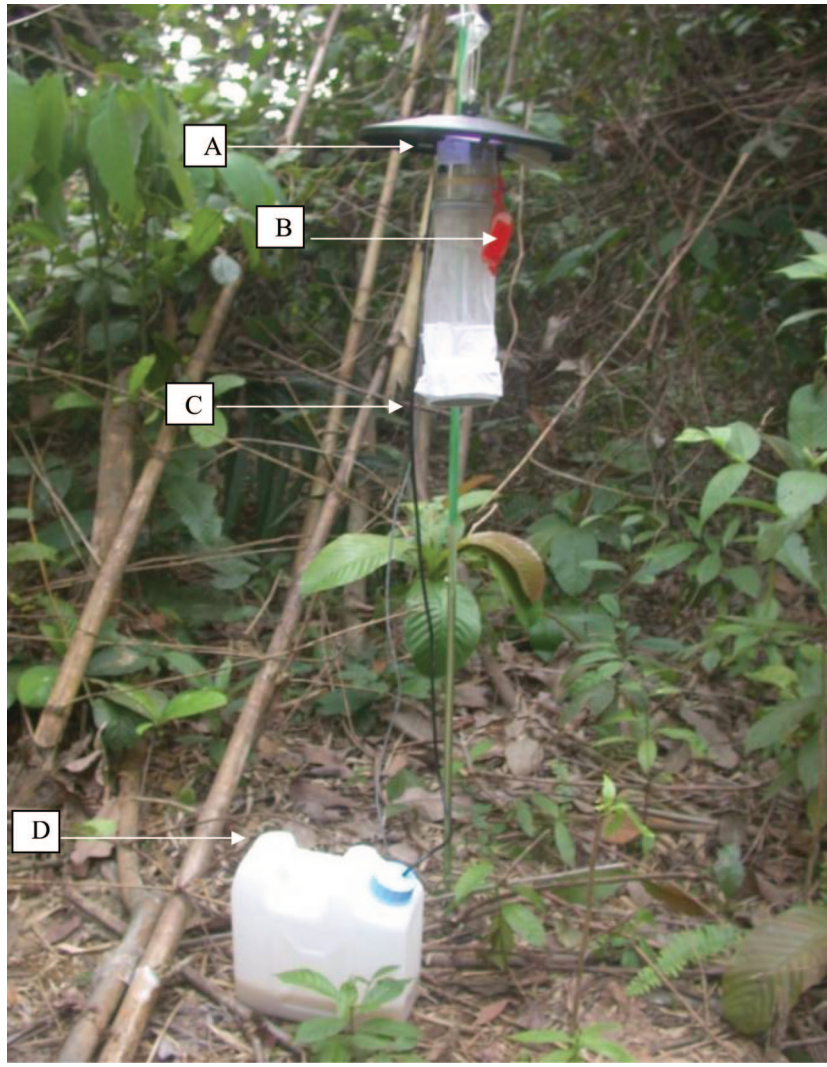


**Fig. 2.** An UV CDC trap (A) baited with a BG lure (B) and $CO_2$ dispersed from a 2-liter plastic jug (C) black plastic tubing (D) containing a mixture of yeast, sugar, and water. (Online figure in color.)

ase chain reaction assay (Scott et al. 1993). The two molecular forms (S and M) of *An. gambiae s.s.* were differentiated using restriction fragment length polymorphism (Fanello et al. 2002).

### Statistical Analysis

A randomized complete-block design with sites as the blocking effect was used to determine capture rates among different trap types. Data were square root (SQRT + 0.5) transformed before statistical analysis, and effects of trap type, trap location (site), and trial (time of year) were evaluated by three-way analysis of variance (ANOVA) using SPSS software v. 11.0.1 (SPSS 2001). Only untransformed data are presented in the text and tables. Statistical analyses were conducted using PROC GLM, and multiple mean comparisons were made using Tukey's multiple range test ($\alpha = 0.05$). Trap malfunctions were recorded as uncollected data and treated as missing values.

## Results

### Species Capture and Infection Rates

In total, 4,788 mosquitoes representing 56 species from nine genera were captured over the course of the study. The following four genera comprised 90% of the total collection and were subsequently analyzed: *Aedes*, *Anopheles*, *Coquillettidia*, and *Culex* (Table 1). The remainder of the collection was excluded from analysis owing to insufficient number of observations. We collected 7 of the 12 anopheline species reported from Liberia (Stojanovich and Scott 1966), of which *An. gambiae* ($n = 149$) comprised 32% (Fig. 1), including a single *An. melas* (Table 2). *An. gambiae s. s.*

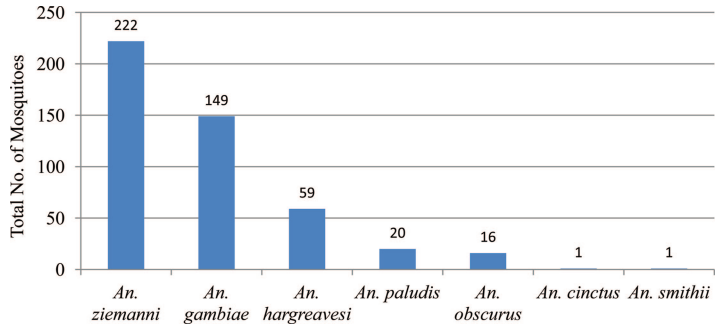


**Fig. 3.** Total number of seven *Anopheles* spp. collected by traps placed at four field sites in Liberia, 2011. (Online figure in color.)

were predominately S-form (86%); however, M (13%) and S/M hybrids (1%) were also captured (Table 2). None of the *An. gambiae* mosquitoes collected tested positive for *Plasmodium falciparum*. *Anopheles ziemanni* Grünberg was the most common *Anopheles* species, comprising 47% of the anopheline capture.

### Trap Efficacy

The UV + Lure + $CO_2$ trap significantly outperformed the LED + Lure trap at capturing *An. gambiae* and captured significantly more *Anopheles* spp. compared with the LED or LED + Lure traps (Table 1). A greater percentage of *An. gambiae* were captured in UV compared with LED trap combinations, 66 and 18%, respectively, with the UV + Lure + $CO_2$ trap capturing the greatest proportion (Table 2). Significantly more *An. gambiae* were captured at site 4 compared with sites 1, 2, and 3 ($F = 18.46$; df = 3, 799; $P < 0.001$). Mean capture rates for *An. gambiae* declined during January (0.01 ± 0.01) and November (0.03 ± 0.06), and increased during March (0.24 ± 0.83), May (0.26 ± 0.10), July (0.25 ± 0.10), and September (0.32 ± 0.06; $F = 3.36$; df = 5, 799; $P < 0.001$).

The UV + Lure + $CO_2$ trap collected more *Coquillettidia* spp. compared with any other trap combination in the study ($F = 9.39$; df = 6, 799; $P < 0.001$) (Table 1). The addition of a lure and $CO_2$ to UV traps did not measurably increase trap captures for any the mosquitoes analyzed, with the exception of *Coquillettidia* spp. (Table 1).

## Discussion

Our study demonstrated an increased attraction response of *An. gambiae* to UV compared with CDC and LED light traps. Although the difference between trap light source was not statistically significant, the UV trap caught about threefold more *An. gambiae s. s.* (0.27 ± 0.07) than the CDC (0.08 ± 0.04) or LED (0.09 ± 0.04) traps. Our results are similar to findings conducted by Service (1970) who documented a greater attraction of *An. gambiae* to UV compared with CDC light traps when placed inside huts. The spectral emission and light intensity of a bulb are important considerations when targeting Afrotropical anophelines (Githeko et al. 1994), and the interpretation of LED traps performance compared with the CDC and UV traps in this study should be guarded. UV fluorescent bulbs emit light from 350 to 360 nm compared with LED UV that emit light at 390 nm, outside of the UV spectra (L. W. Cohnsteadt, personal communication). Moreover, the UV bulbs use 590 mA compared with 160 mA for the LED and are at least four times brighter than the LED. However, LEDs offer many advantages over conventional bulbs. Unlike incandescent bulbs, traps with LEDs can be interchangeable, potentially offering selectivity of a particular bandwidth or color to attract a specific mosquito species (Cohnstaedt et al. 2008). Moreover, LEDs are energy efficient, durable under field conditions, are less fragile for carrying, and seldom need

**Table 1. Number (mean ± SE) of mosquitoes caught during field experiments using three traps with lure and $CO_2$ combinations placed at four sites in Liberia, 2011**

| Mosquito species/genera | Traps[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | CDC | LED | LED + Lure | LED + Lure + $CO_2$ | UV | UV + Lure | UV + Lure + $CO_2$ | *F* | *P* |
| *An. gambiae s. s.* | 0.08 ± 0.04abc | 0.09 ± 0.04abc | 0.03 ± 0.02a | 0.22 ± 0.08c | 0.27 ± 0.07bc | 0.27 ± 0.07bc | 0.33 ± 0.10c | 3.69 | 0.001 |
| *Anopheles* spp. | 0.55 ± 0.10abc | 0.28 ± 0.07a | 0.23 ± 0.06a | 0.54 ± 0.12abc | 0.64 ± 0.13abc | 1.0 ± 0.23bc | 0.99 ± 0.16c | 6.76 | <0.001 |
| *Aedes* spp. | 0.09 ± 0.27a | 0.06 ± 0.03a | 0.09 ± 0.04a | 0.14 ± 0.04ab | 0.14 ± 0.04ab | 0.22 ± 0.05ab | 0.33 ± 0.10b | 3.48 | 0.002 |
| *Coquillettidia* spp. | 1.08 ± 0.30ac | 0.53 ± 0.22ab | 0.23 ± 0.06ab | 0.57 ± 0.16ab | 1.69 ± 0.42c | 1.71 ± 0.43c | 2.59 ± 0.56d | 9.39 | <0.001 |
| *Culex* spp. | 2.43 ± 0.44abc | 2.04 ± 1.10a | 1.90 ± 0.90a | 1.52 ± 0.30a | 2.83 ± 0.58abc | 3.86 ± 1.33b | 4.56 ± 1.90b | 3.97 | 0.001 |
| Total mosquitoes | 4.93 ± 0.77ab | 3.40 ± 1.10a | 3.33 ± 1.15a | 4.08 ± 0.77ab | 7.05 ± 1.02bc | 9.70 ± 2.03c | 9.91 ± 2.20c | 11.18 | <0.001 |

Lures were BG-lure and replaced after every 5-d trial. $CO_2$ was manufactured from combining yeast, sugar, and water.

[a] $n = 120$ trap nights per trap. Means within each row followed by the same letter are not significantly different ($P > 0.05$), Tukey's mean separation applied to SQRT + (0.05) transformed.

**Table 2. Number of *Anopheles gambiae s. l.* collected by traps placed at four field sites in Liberia, 2011**

| Traps | *An. melas* | *An. gambiae s.s.* | | | Total (%) |
|---|---|---|---|---|---|
| | | S-form | M-form | S/M-form | |
| CDC | 0 | 9 | 0 | 0 | 9 (5.4) |
| LED | 0 | 9 | 1 | 0 | 10 (6.0) |
| LED + Lure | 0 | 2 | 1 | 0 | 3 (3.3) |
| LED + Lure + $CO_2$ | 1 | 21 | 3 | 1 | 26 (19.0) |
| UV | 0 | 28 | 4 | 0 | 32 (19.2) |
| UV + Lure | 0 | 27 | 3 | 0 | 30 (22.2) |
| UV + Lure + $CO_2$ | 0 | 32 | 6 | 0 | 39 (25.3) |
| Total | 1 | 128 | 19 | 1 | 149 |

to be replaced compared with incandescent and UV light bulbs.

With the exception of the LED + Lure + $CO_2$ trap, the addition of the BG lure and $CO_2$ did not significantly increase capture rates of *An. gambiae s.s.* or *Anopheles* spp. (Table 1). We were unable to accurately measure the rate of $CO_2$ discharge from our traps; however, robust fermentation did occur in the chambers, and we used the exact amounts of yeast, sugar, and water according to Smallegange et al. (2010) who reported an average of 220.2 ± 50.1 (ml/min ± SD) for 10 h under field conditions. Moreover, Saitoh et al. (2004) reported $CO_2$ production of 40.6 ± 2.1 ml/min after 10 h using a similar style setup. Regardless of the amount of $CO_2$ emitted, we attribute the lack of significance among treatments to traps being placed away from human dwellings where volatile lure odors were less likely to be concentrated.

The UV + Lure + $CO_2$ did significantly capture more *Anopheles* spp. compared with LED + Lure traps (Table 1). It is also possible that UV light was a greater visual cue over olfactory ones, essentially over-riding any increased attraction from the lure or $CO_2$. *An. gambiae s.s.* can discern between multiple hosts, and peripheral receptors capable of detecting specific semiochemicals on the human skin are believed responsible for detection of and subsequent location of a human bloodmeal (Costantini et al. 1999, Takken and Knols 1999). A combination of ammonia, carboxylic, and lactic acids mimic skin odors, resulting in a tripartite synergistic effect on the host-seeking behavior of *An. gambiae s.s.* (Smallegange et al. 2005, Okumu et al. 2010). Carbon dioxide also serves as an important synergist with other semiochemicals. For example, inside a Kenyan greenhouse, *An. gambiae* were captured significantly more in MMX-traps baited with $CO_2$, human foot odor, and ammonia compared with $CO_2$ alone (Njiru et al. 2006), and Murphy et al. (2001) demonstrated lactic acid–$CO_2$-baited traps placed inside huts captured significantly more *An. gambiae* and *Anopheles funestus* Giles compared with traps baited with $CO_2$ only. However, only olfactory cues were being evaluated in these studies and therefore, lights were either removed or were not used. The addition of $CO_2$ to UV traps did increase the overall capture of *Coquillettidia* spp. (Table 1). Similarly, yeast-generated $CO_2$ traps captured a substantial number of *Aedes albopictus* (Skuse) and *Culex pipiens* L., in Japan, but no *Anopheles* spp. were collected (Saitoh et al. 2004).

Peters (1956) documents 84 mosquito species from Liberia during a survey conducted in 1953–1954, as well as studies from the early 1930s. We did not observe all of these species during our study. Of particular significance is the absence of *An. funestus* in our trap collections. However, while this species has been recorded in Liberia, Peters (1956) reported the population density to be greater inland and Fox (1958) confirmed it to be rare, especially along the coast. We also did not capture *An. Arabiensis*, and although this species has been documented in neighboring Gambia, the distribution of this member is focused in areas with lower rainfall or drier savannah areas (Coetzee et al. 2000). The only other member of the *An. gambiae* complex captured in our study was *An. melas*, a brackish water species known to develop in semimonthly spring-tide pools (Fox 1958).

*An. ziemanni* encompassed almost one-half of the anopheline capture (Fig. 3). Similarly, Qiu et al. (2007) in the Gambia, determined this species comprised a significant portion of anopheline captured in traps, and although not a human malaria vector, *An. ziemanni* will readily feed on humans, but prefers other animals, such as goats (Fox 1958, Stojanovich and Scott 1966). Our traps may have targeted this species over *An. gambiae*, due in part to $CO_2$, which is known to attract more zoophilic and opportunistic anopheline species than anthropophilic ones (Dekker and Takken 1998).

The majority of the *An. gambiae s.s.* captured in our study were identified as S-forms (86%; Table 2) and are known to breed mostly in rain-dependent pools across sub-Saharan Africa (Caputo et al. 2011). The capital of Liberia (Monrovia) receives 462 cm of annual precipitation, with the greatest amounts occurring from July to September (Climatemps 2012). Wet periods provide ideal conditions for *An. gambiae* to flourish, and one would expect the maximum number of available breeding sites to occur during the wet season. Indeed, we collected the greatest number of *An. gambiae* during July and September, whereas few were collected in January when monthly precipitation averages 79, 72, and 5.1 cm, respectively (Climatemps 2012). The inter-form cross (S/M) represented 1% of our capture, and Caputo et al. (2011) reports this as typical and consistent with similar findings from other West African countries.

Across the study sites, we caught few *An. gambiae* ($n$ = 149). However, traps were placed away from human habitation, and *An. gambiae* capture rates are known to increase when distance from traps to human dwellings decreases. Although we did not place traps inside homes, the disparity between indoor and outdoor light trap captures is best illustrated by Joshi et al. (1975) who demonstrated a 10-fold increase of *An. gambiae* collected in traps placed inside compared with outside. Additionally, Odetoyinbo (1969) reported a similar outcome when only 197 *An. gambiae* specimens were collected from traps placed in an open field compared with 1,442 specimens collected from traps placed inside a room.

It is not surprising that none of our *An. gambiae* specimens tested positive for *P. falciparum.* Our study placed light traps in the field, thereby targeting host-seeking nonparous mosquitoes. Light traps have captured significant quantities of *P. falciparum*-positive *An. gambiae*, in some cases even more than human-biting collections (Mbogo et al. 1993). However, Mboera (2005) attributed this increase to traps being strictly placed inside human dwellings; thereby attracting and capturing predominately resting mosquitoes that are known to yield higher sporozoite rates. Future studies focusing on the malaria entomological inoculation rate should focus placing traps inside human shelter opposed to open field sites, as this would not only increase the trap yields of anthropophilic species, but would target a population likely to have taken a bloodmeal.

Synthetic blends of ammonia, lactic acid, and carboxylic acids create a synergistic effect that *An. gambiae s.s.* find attractive (Smallegange et al. 2005). Although no lure is as effective for attracting mosquitoes compared with the human body and landing catches remain the gold standard for determining malaria mosquito infection rates (Mboera 2005), future development of lures in the form of visual and odor attractants to target *An. gambiae* remains paramount. For surveillance purposes, lures would provide a consistent degree of attraction, reducing operator variation that occurs while conducting human landing counts and potential health risks. For control measures, attractants could be used for a myriad of applications. First, attractants may be used during mass trapping, whereby traps are used to reduce the mosquito population to sustainable levels; second, to augment other malaria reduction initiatives such as push–pull strategies, whereby mosquitoes are lured into traps rather than human dwellings; third, attractants may serve as the primary bait attractants in lethal outdoor targets containing insecticides (Okumu et al. 2010). Recent studies have shown attractant-baited "kill stations" are effective when placed between mosquito larval habitats and human dwellings and may complement already existing malaria control programs (Sumaye et al. 2012). Finally, understanding the olfaction and behavioral response of *An. gambiae* to human-specific attractants may assist in future development of mosquito repellents. One of the major challenges to developing good repellents is a lack of understanding how compounds interact and synergize with each other, as well as their mode of action on the physiological and behavioral level of specific mosquitoes (Costantini et al. 1999).

In conclusion, our study demonstrated that UV light collected the most *An. gambiae* although the addition of lures and $CO_2$ to the traps did not significantly increase the number collected. The addition of these attractants should still be considered, especially for indoor surveillance. Moreover, the use of yeast, sugar, and water combination should be considered as an attractant with light traps when dry ice or compressed gas is unavailable or expensive.

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